THE DEVELOPMENT OF THE ASCOCARP OF LACHNEA SCUTELLATA[1]

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(WITH PLATE IX AND FIFTY-ONE FIGURES)

The material upon which the present study is based was collected at Cold Spring Harbor, Long Island, where the ascocarps of *Lachnea* were found in large numbers upon decaying wood in damp places. The ascocarps appear to be frequently produced in crops, as a considerable number of about the same age are often found on a single log. If all of these are removed while still young, a second crop will usually appear in a few days. If now the young ascocarps are removed as they appear, successive crops may continue to be produced for some time. By this means a large number of young stages can be quite easily obtained.

For microscopical study, sections were cut 3–5 μ thick and stained with Flemming's triple or Haidenhain's iron-alum hematoxylin. The latter gave the best results.

Lachnea scutellata has a disk-shaped ascocarp, 2 mm.–1 cm. in diameter, the upper surface of which is covered by the hymenium, which is colored red. The margin and lower surface of the disk are brown and thickly beset with long brown setae. The setae are long, septate hyphae, the outer walls of which are greatly thickened. A cross-section of an ascocarp (plate fig. 1) shows that the inside is composed of densely interlacing hyphae, while the margin and lower surface are covered by a parenchymatous cortical layer consisting of large, thick-walled hyphae which run nearly parallel to each other and perpendicular to the outer surface of the ascocarp. WORONIN (**38**) described the ascocarp of *Lachnea scutellata* as originating in the production of an archicarp, which soon became surrounded by vegetative hyphae that obscured its further development.

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In the youngest specimens obtained, the archicarp consisted of a row of 7–9 cells, which had just become surrounded by vegetative hyphae. The ascogonium is the penultimate cell of the archicarp, which when mature consists of about 9 cells (plate fig. 3). The ascogonium and all of the vegetative cells are multinucleate. In the youngest specimens the ascogonium was about one-third to one-fourth its size at maturity. There was observed neither at this time nor later any sign of an antheridium, and since in the young specimens the ascocarp consisted of only a few hyphae, it should have been plainly visible even if degenerated. It seems probable, therefore, that no antheridium is present.

Before the ascogonium reaches its mature size, the walls of the vegetative hyphae on the outside of the young ascocarp become thickened, and these hyphae form the outer covering of the ascocarp (plate fig. 2). This covering undergoes no further growth, but remains at the base of the mature ascocarp and forms the first part of the cortex. The hyphae around the ascogonium remain active and give rise, over the ascogonium, to small hyphae which grow out to form paraphyses (plate fig. 3). The same hyphae which give rise to the hyphae producing the paraphyses give off branches, around the region of the paraphyses, some of which grow up and add to the cortex, while others grow out and form setae. As the cells of the setae and cortex reach their mature size, they become greatly vacuolated and the outer walls increase greatly in thickness. When the setae are first formed, they are bent down toward the center of the top of the young ascocarp, and thus form a covering over the developing hymenium (plate fig. 4). When a part of the cortex is once formed, the development of that part ceases, and further additions are made only in the region between the paraphyses and the cortex. The hyphae here remain active and give rise on one side to paraphyses and on the other to setae and more of the cortex of the ascocarp. As this continues, the older setae are carried outward, and finally come to be on the lower surface of the ascocarp. The setae which are formed first are not as long as those which are formed later, so that the setae around the margin of the disk are longer than those on the under surface. As the hymenium increases in diameter, by the production of more

paraphyses and the pushing in of the ascogenous hyphae, which by this time have grown out from the ascogonium, it becomes too large to be covered by the setae and is thus exposed. When this has occurred, the ascocarp has attained its mature form (plate fig. 1). The relation of the various parts of the ascocarp is shown diagrammatically in fig. 1. In this diagram are shown both the ascogonium and asci, whereas the ascogonium always disappears before the formation of asci.

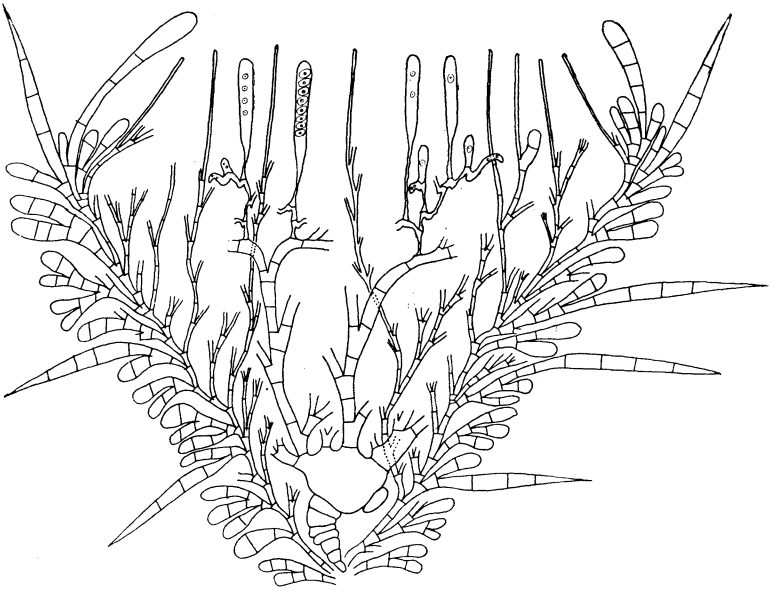
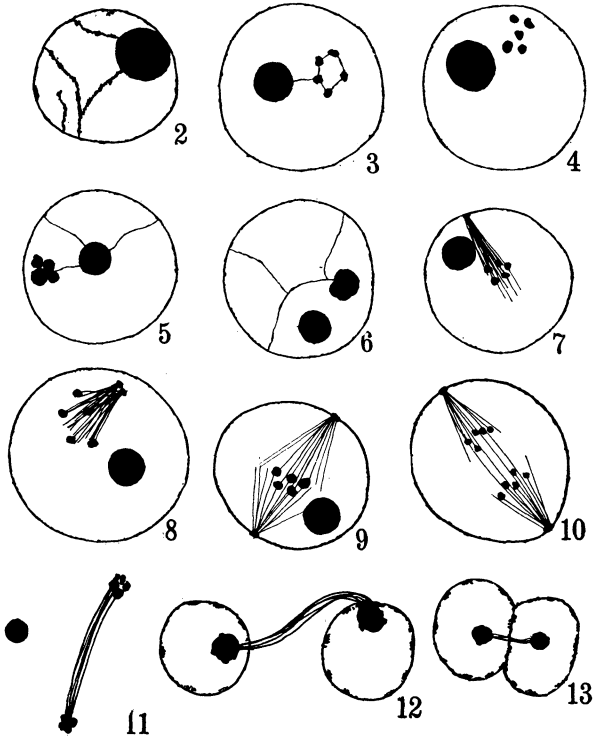


FIG. 1.—Diagrammatic cross-section of ascocarp

The vegetative nuclei usually contain a nucleolus and a small amount of scattered chromatin, but sometimes the chromatin is collected into a rounded mass resembling a nucleolus. In dividing the vegetative nuclei show five chromosomes. The nuclei of *Lachnea* contain comparatively little stainable material, as will be seen from the figures. This scarcity of stainable material makes the figures appear diagrammatic. Such is not the case, however, as all figures were drawn with a camera lucida, and in those illustrating nuclear details, all of the stainable material in the nuclei is

figured. In most cases the cytoplasm is omitted, as this resembles that usually found in Ascomycetes.

The nuclei in the ascogonium resemble the vegetative nuclei



FIGS. 2–13.—Fig. 2, nucleus of ascogonium in resting stage; fig. 3, formation of chromosomes in nucleus of ascogonium; figs. 4–6, arrangement of chromosomes when first found in nucleus of ascogonium; fig. 7, formation of spindle in nucleus of ascogonium; fig. 8, division of centrosome in nucleus of ascogonium; the centrosomes are on the nuclear wall, but owing to their position appear to be inside the nucleus; fig. 9, metaphase in nucleus of ascogonium; fig. 10, anaphase in nucleus of ascogonium; fig. 11, telophase in nucleus of ascogonium; fig. 12, reorganization of nuclei in ascogonium; fig. 13, reorganization of nuclei, in contact, in ascogonium; all ×11,200.

except that they are somewhat larger. The chromatin is usually scattered throughout the nucleus, but sometimes it is arranged in a definite spireme (fig. 2). This condition probably indicates the approach of division. It has not been possible to determine defi-

nitely whether or not the spireme is continuous. Often several loops are tangled together, so that it is impossible to follow individual parts. Still more frequently parts of the spireme run along the nuclear membrane for considerable distances, so that even if it were continuous it could be followed only with considerable difficulty. At other times there appear to be definite breaks. This appearance may be due to a failure of the spireme to take the stain or to poor fixation, but there is nothing to indicate that such is the case. The spireme, soon after its formation, appears to contract and divide to form five chromosomes (figs. 3, 4). The chromosomes may be rather widely separated (fig. 4), but frequently they are collected together into a compact group resembling a second nucleolus (figs. 5, 6). The group can be distinguished, however, from a nucleolus by its irregular outlines. This grouping of the chromosomes is not confined to the ascogonium, but can be seen throughout the ascogenous hyphae and in the prophases of the second and third divisions of the ascus. It is probably also the explanation of the grouping of the chromatin seen in the vegetative nuclei.

While the chromosomes are being formed, linin fibers make their appearance in the nucleus. At the same time a centrosome appears on the nuclear membrane. This was not visible during the resting condition and appears to arise *de novo*. The centrosome is not a point, but rather a flattened area, apparently composed of many granules. When the centrosome was first observed, it was already connected with the chromosomes by the linin fibers in the nuclear cavity (fig. 7). Soon after this the centrosome (fig. 8) divides, and the daughter centrosomes move apart and come to be situated at the opposite poles of the complete spindle (fig. 9). The centrosomes in fig. 8 are against the nuclear membrane, but owing to their position appear in the figure to be within the nucleus. The five chromosomes then divide and five daughter chromosomes proceed to each of the opposite poles (fig. 10). The nuclear membrane now breaks down, and the two groups of chromosomes and the nucleolus, which soon disappears, are left free in the cytoplasm (fig. 11). The two groups of chromosomes are usually separated far enough so that when they reorganize the daughter nuclei are separated by an appreciable distance (fig. 12). Frequently, how-

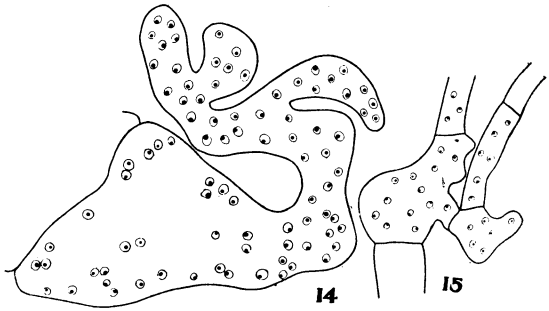
ever, the daughter nuclei reorganize so close together that after a slight growth they are pressed against each other and resemble fusing nuclei (fig. 13). The spindle fibers are frequently present at this stage, and can be seen connecting the two masses of chromatin, which are still visible in the daughter nuclei. Frequently the masses of chromatin lie against the nuclear membrane, and the disappearing fibers are entirely outside the nucleus, but at other times the fibers appear to cross the nuclear cavity, as in fig. 13. Frequently the chromatin appears, at first sight, to be in the center of the nucleus, when in reality it is lying against the membrane. This is due, of course, to the fact that the chromatin is at the upper or lower surface of the nucleus as it is viewed from above.

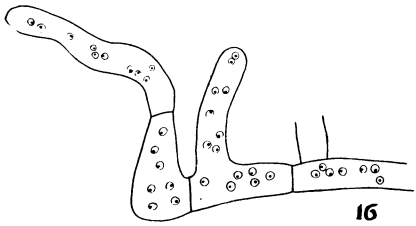
Division seems to take place rapidly throughout the growth of the ascogonium and the development of the ascogenous hyphae. The nuclei do not divide simultaneously, as all stages, including resting nuclei, can be found in a single ascogonium. There appear, however, to be periods in which division takes place, followed by others in which all of the nuclei are in the resting condition, for a large number of divisions are frequently found in a single ascogonium, while others show only resting nuclei. The same type of division that has just been described and the same number of chromosomes persist throughout the development of the ascogonium and ascogenous hyphae. The nuclei decrease somewhat in size during the growth of the ascogonium, and in the early stages of the development of the ascogenous hyphae, but as the ascogenous hyphae develop further, the nuclei increase in size until they come to be slightly larger than in the young ascogonium.

No fusion of nuclei has been observed in the ascogonium or in the ascogenous hyphae except in the tips where two nuclei fuse to form the primary nucleus of the ascus. A number of cases were seen in which two nuclei were pressed against each other, but in all of these the nuclear membrane was intact between the nuclei, and the appearance seemed to be due to the fact that the nuclei, after division, had reorganized close together, in the manner previously described. It may be said that a fusion of the nuclei would be hard to find, but they have been looked for very carefully in a large number of well fixed and stained preparations. The slight

decrease in the size of the nuclei during the development of the ascocarp and the persistence of the same number of chromosomes throughout the ascogonium and ascogenous hyphae, moreover, indicate very strongly that a fusion of nuclei during this stage is not to be expected.

When the ascogonium has reached its mature size, it gives off a number of large ascogenous hyphae which are multinucleate from the first (plate fig. 3). The nuclei do not appear to be arranged in pairs or in any other definite manner, but to be scattered irregularly in the hyphae (fig. 14). They are undergoing division rather rapidly, as has been previously described. About this time the cytoplasm and nuclei of the other cells of the archicarp begin to degenerate. These cells apparently do not fuse together as in *Ascophanus carneus* (CUTTING **7**). The ascogenous hyphae grow up among the vegetative hyphae which are situated over the ascogonium and have been mentioned as giving rise to paraphyses. As the ascogenous hyphae increase in length, they branch freely and become divided up into a number of large multinucleate cells. Some nuclei are left in the ascogonium and these finally degenerate. When the ascogenous hyphae are growing out from the ascogonium, the vegetative cells over the ascogonium (plate fig. 3) are slender, densely protoplasmic, and extend upward toward the covering of the ascocarp. They thus have the appearance of young paraphyses, but do not take part in the formation of the hymenium until they have developed further. As they grow up they branch freely and





FIGS. 14–16.—Fig. 14, outgrowth of ascogenous hyphae from ascogonium; fig. 15, storage cells giving off paraphyses; fig. 16, tips of ascogenous hyphae in hymenium; all ×525.

become thicker and less densely protoplasmic. As the developing ascogenous hyphae grow up and branch among these vegetative hyphae, the older parts of the vegetative hyphae cease to have the appearance of paraphyses, while the younger parts still form a layer ahead of the ascogenous hyphae. When the place where the hymenium is to be formed is finally reached, the layer of paraphyses is thus already completely developed (plate fig. 4). The continued upward growth and branching of the vegetative and ascogenous hyphae causes the hymenium to have a much greater diameter than it would have had if it had been formed before the branching had taken place. Some of the vegetative hyphae in the subhymenial layer give off branches which form large, densely staining storage cells. These in turn give rise to more paraphyses (fig. 15). In a few cases nuclei in these storage cells have been seen to be fusing, and since in some cases the fusing nuclei are exceptionally large, it may be that nuclei which have been formed by fusion may themselves fuse. The fusion of nuclei in the storage cells is of regular occurrence in *Leotia* (BROWN **6**), but is probably exceptional in *Lachnea scutellata*, as most of the nuclei in the storage cells of this species are small and of nearly uniform size.

While the storage cells are being formed in the subhymenial layer, the ascogenous hyphae can be seen, in the same region, as rows of large multinucleate cells. These give off smaller multinucleate branches which extend upward into the lower part of the hymenium (fig. 16). It is from these branches that the asci are to be formed. The tips of these branches frequently contain two nuclei, and it seems probable that these are cut off together in a single cell, as no such uninucleate cells have been observed in the hymenium or subhymenial layer, although binucleate cells are of frequent occurrence. It is, of course, still possible that uninucleate cells may sometimes be cut off, and that these may have been overlooked, as the uninucleate condition would probably last only a short time. The cutting off of two nuclei in the tip of an ascogenous hypha has been described by MCCUBBIN (**28**) in *Helvella elastica*. The cutting off of two nuclei or a single one, which subsequently divided, in *Lachnea scutellata* would probably not have any effect on the further development, since, as has already been

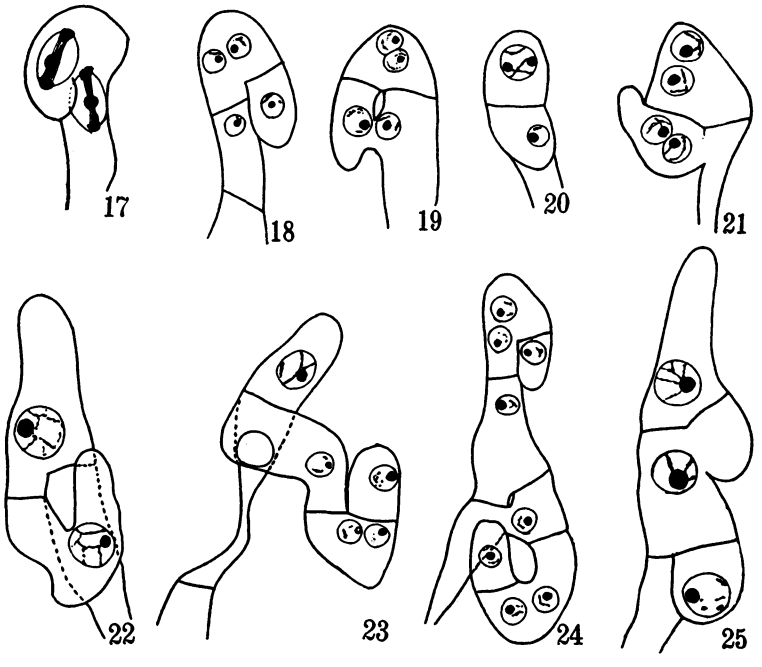
described, the nuclei undergo division in the ascogenous hyphae, so that the two nuclei which are in the tip of a hypha are probably closely related. There appears, moreover, as has been previously pointed out (BROWN **6**), to be no reason for thinking that the relation of fusing nuclei can make any difference, if these are all in the same plant and are derived from a single nucleus, with the haploid number of chromosomes.

The nuclei in those cells of the ascogenous hyphae which are below the hymenium finally degenerate. In doing so they often swell up to several times their original size, after which the nuclear membrane gradually disappears. This process is quite similar to that described by HARPER (**22**) for the nuclei in the trichogyne of *Pyronema confluens*. Before degenerating two or three of the nuclei sometimes fuse together. Such fusions are not confined to the nuclei of the ascogenous hyphae, but may occur in other degenerating cells.

The binucleate cells previously described as being formed from the ascogenous hyphae grow up in the hymenium and bend over at the tip. The two nuclei pass into the bent portion and divide in the same manner that has been described for the nuclei in the ascogonium (fig. 17). At metaphase there are five chromosomes, and at anaphase five pass to each pole. Walls come in between the daughter nuclei of each pair, thus forming a binucleate penultimate and a uninucleate ultimate and antipenultimate cell (fig. 18). This is of course a typical hook. The two nuclei in the penultimate cell may fuse to form the nucleus of an ascus (fig. 20), but often they divide and give rise to the nuclei of another hook (fig. 24). The ultimate cell usually grows down and fuses with the stalk (fig. 19), after which the nucleus from the stalk usually migrates into the ultimate cell (fig. 21), although occasionally the nucleus of the ultimate cell may pass into the stalk. After the nucleus of the stalk has migrated into the ultimate cell, it may fuse with the nucleus of the ultimate cell to form the primary nucleus of an ascus (fig. 22), but usually the two nuclei divide and the ultimate cell grows out to form another hook (figs. 23, 24). Sometimes the nucleus formed by the fusion of the nuclei of the ultimate and antepenultimate cells does not develop further. This is usually asso-

ciated with a vacuolated condition of the cytoplasm. Fig. 25 shows a case in which the penultimate cell has developed into a second hook. The nuclei of the ultimate and antepenultimate cells have fused, but the fusion nucleus has not developed further. The penultimate cell of the second hook has given rise to an ascus, while the nucleus of the ultimate cell has migrated into the antepenultimate and fused with its nucleus.

The processes described above, by which either the ultimate



FIGS. 17–25.—Fig. 17, tip of ascogenous hyphae, showing form of hook and division of nuclei; fig. 18, binucleate penultimate and uninucleate ultimate and antepenultimate cells; fig. 19, fusion of nuclei in penultimate cell and fusion of ultimate and antepenultimate cells; fig. 20, fusion nucleus in antepenultimate cell; fig. 21, migration of nucleus from antepenultimate to ultimate cell, followed by outgrowth of ultimate cell; fig. 22, formation of asci from both ultimate and antepenultimate cells; fig. 23, formation of hook from ultimate cell and ascus from penultimate; fig. 24, formation of hooks from both ultimate and penultimate cells; fig. 25, case in which nucleus from antepenultimate cell migrated into ultimate and fused with nucleus of ultimate; a hook was formed from binucleate penultimate cell, the penultimate cell of which in turn gave rise to an ascus, while the nucleus of the ultimate cell migrated into the antepenultimate and fused with its nucleus; all ×1400.

or antepenultimate cell may give rise to a hook, may be repeated many times, so that a large number of asci may be formed finally from a single hypha. Even in young ascocarps, five or six hooks may frequently be seen joined together in various ways, and if it were possible to follow a hypha for a considerable distance, the above number would of course be greatly increased.

The significance of these phenomena has been discussed in a previous paper on *Leotia* and *Geoglossum* (BROWN **6**), in which genera they also occur.

As new hooks are successively developed from older ones, that part of the ascogenous hypha which connects the successive hooks, as well as the older parts of the hypha, become vacuolated to such an extent that no cytoplasm can be seen in them. Despite this fact, new hooks and asci are formed quite rapidly. It seems probable, therefore, as HARPER (**22**) suggests, that the developing asci obtain their nutrient material from the paraphyses, which are in contact with them, by transfusion through the walls.

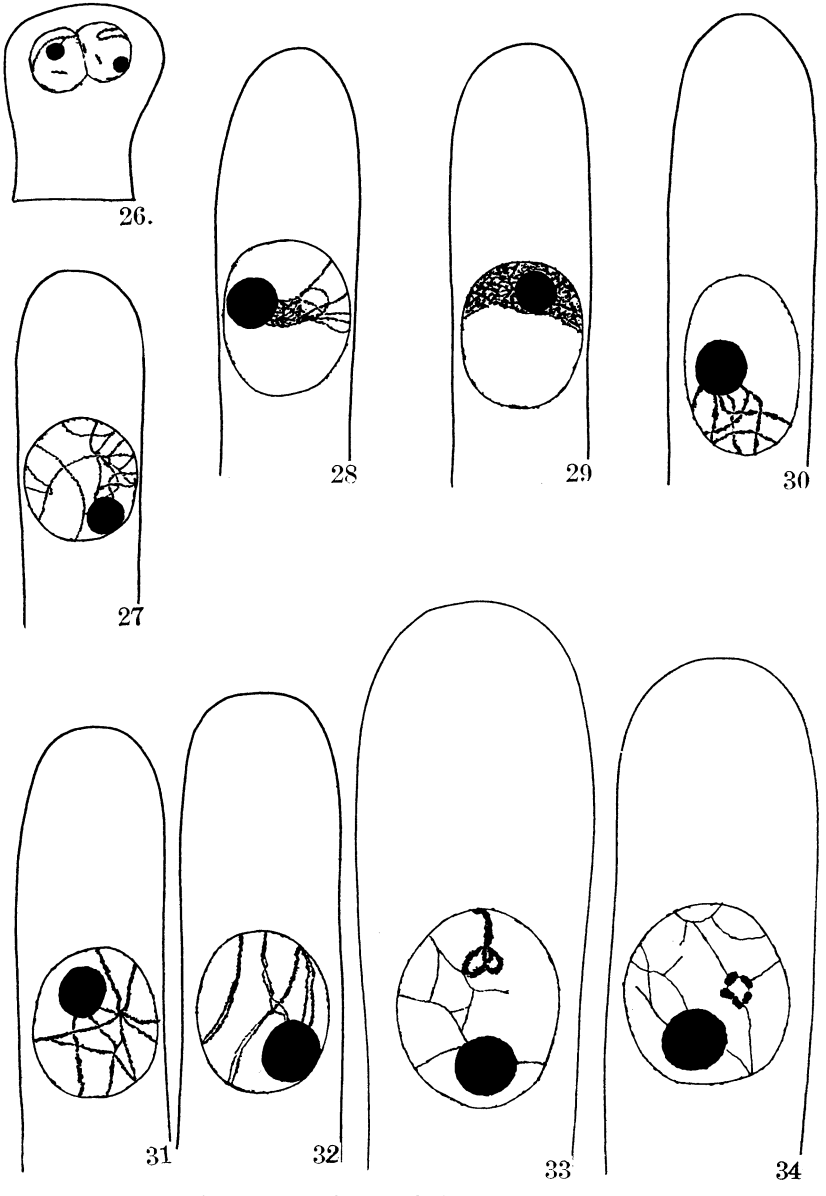
The multiplication of the number of hooks gradually raises the level at which asci are formed. At the same time, the level at which the paraphyses come off is also raised by the formation of new ones from the basal portion of older ones and from storage cells which are being continually formed at a higher level. As growth continues and the hymenium rises higher and higher, the subhymenial layer is increased in height by the addition of the older parts of the hymenium, which are gradually left behind.

While the hymenium is thus being raised, it also increases in diameter. As has already been described, the cells between the hymenium and cortex continually produce new cells which give rise to paraphyses around the margin of the hymenium. At the same time, hooks formed from the ultimate or penultimate cells of older ones grow in among the paraphyses. Owing to the processes described above, an ascocarp, after it assumes its mature form, may increase greatly in both height and diameter.

When the two nuclei which fuse to form the primary nucleus of the ascus are in the process of fusion, they contain comparatively little chromatin. This is scattered somewhat irregularly on linin fibers, but shows an approach to the spireme condition

(fig. 26). The fusion nucleus grows rather rapidly, and as this continues the chromatin soon comes to be arranged in a definite, fine spireme (fig. 27). When this condition has been reached, the spireme does not usually show any free ends, and it can frequently be traced as a continuous thread for considerable distances. It is impossible, however, to follow it through some of the tangles. Frequently threads run to the nuclear membrane or nucleolus, after which it is not possible to trace them further. This suggests that the spireme is not continuous throughout its entire length, but this conclusion must be considered doubtful, as it is difficult to follow a spireme along the nuclear membrane, which is usually irregularly thickened, or to distinguish it from the nucleolus when it is in contact with the latter. While the nucleus is still far from its final size, the spireme shows the approach of synizesis by beginning to collect in a tangle either around or to one side of the nucleolus (fig. 27). This usually continues until all of the spireme is arranged in a dense tangle in which little detail can be seen (fig. 29). No evidence of a fusion of spiremes during this stage was observed. An examination of figs. 27 and 28 will show that the spireme is not double as it goes into synizesis. The spireme was occasionally seen contracted into a mass about as dense as the nucleolus. This extreme condition may have been due to fixation, but the regular occurrence of synizesis at this stage, and in material in which the fixation seemed to be perfect, certainly seems to indicate that synizesis is, as MOTTIER (**29**) thinks, a stage in development, and not an artifact due to fixation, as is claimed by SCHAFFNER (**32**). This view is supported by the fact that the spireme is quite different in appearance before and after synizesis. Synizesis probably lasts for a considerable time, as the nucleus and ascus grow considerably during this period.

At the end of synizesis the spireme, which is now much thicker than before, loosens up and becomes spread through the nucleus (figs. 30 and 31). The continuity of the spireme throughout its length at this stage is, just as before synizesis, doubtful. After the spireme has become spread through the nucleus, it splits longitudinally (fig. 32). This splitting appears to extend through almost if not quite the entire length of the spireme. The two halves,



FIGS. 26–34.—Fig. 26, fusion of two nuclei in ascus; fig. 27, early stage in approach of synizesis in nucleus of ascus; fig. 28, later stage in approach of synizesis; fig. 29, synizesis in nucleus of ascus; fig. 30, spireme just after synizesis; fig. 31, spireme spread through nucleus; fig. 32, split spireme; fig. 33, contracted spireme just before formation of chromosomes; linin fibers apparent; fig. 34, nucleus with five chromosomes and well developed fibers; all ×2800.

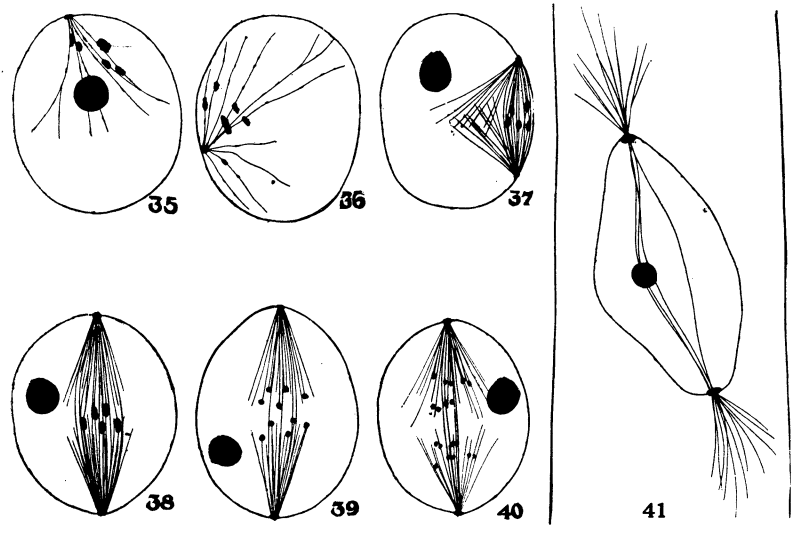
however, soon come together again, after which all traces of the split are usually lost, although sometimes evidences of it may be apparent even after the formation of the chromosomes.

After the two halves of the spireme have come together, it begins to contract. This contraction continues until the spireme shortens very considerably (fig. 33). The spireme at this stage has the appearance of a continuous thread, the ends of which are probably free. The spireme finally segments into five somewhat elongated chromosomes (fig. 34). Each of these chromosomes is probably bivalent, since the nucleus received five chromosomes from each of the two nuclei which by fusing gave rise to it. The bivalent condition, however, is not indicated by the form of the chromosomes. In this they are probably similar to those of most plants. In *Peperomia* (BROWN **4**), however, the two halves appear during the heterotypic prophase, as separate chromosomes connected by linin fibers; while in *Oenothera* (GATES **17**) the diploid number of chromosomes appears at the same stage, and in this case some of the chromosomes may not be arranged in pairs.

As the spireme contracts, linin fibers appear within the nucleus (fig. 33). Along those fibers, and especially in the early stages, there are small granules which have the appearance of chromatin. They usually stain less densely than the chromatin of the spireme, but frequently they are large and numerous enough to make the fibers along which they are scattered resemble the spireme. It was not possible to tell whether the substance of these granules passed to the chromosomes or took part in the formation of more linin fibers, but since as they disappear the number of linin fibers increases considerably, it seems probable that part of the granules take part in the formation of the fibers. No evidence of the formation of these fibers from the linin of the spireme by the migration of the chromatin has been observed, but since the continuity of the spireme in the early stages is doubtful, and these fibers may resemble the spireme very closely, such a possibility, while not probable, can hardly be said to be excluded. It is certain, however, that most of these fibers which will later on take part in the formation of the spindle are formed *de novo*.

As the spindle fibers increase in number, they become connected

with a centrosome which makes its appearance on the nuclear membrane, and some of them connect the centrosome with the chromosomes (fig. 35). No signs of this centrosome have been visible up to this time, and as there is nothing to indicate that it persists through the resting stages, it is probable that it is formed *de novo* at each division. In this respect it resembles the centrosphere-like bodies in *Polysiphonia violacea* (YAMANOUCHI **39**), the centrospheres in *Corallina* (DAVIS **11**), and the kinoplasmic caps



FIGS. 35–41.—Fig. 35, fibers attached to centrosome; fig. 36, nucleus showing extra body which appears much like a chromosome; fig. 37, late prophase of first division in ascus; fig. 38, metaphase of first division; fig. 39, early anaphase of first division; fig. 40, late anaphase, showing division of daughter chromosomes; fig. 41 telophase of first division; all ×2800.

in *Griffithsia bornetiana* (LEWIS **25**). Deeply staining granules are frequently present in the cytoplasm of *Lachnea*. These are particularly abundant around the nucleus at this division. The nuclear membrane does not have an even appearance, but is irregularly thick, and often the granules just described are in contact with it. Owing to these facts it has not been possible to trace the origin of the centrosome. The centrosome here, as in the divisions previously described, is not a spherical body, but a flattened structure composed of a number of granules.

When the five chromosomes have become connected with the centrosome, other deeply staining bodies are frequently present on the linin fibers. These are usually small and are probably similar to the granules previously described. Sometimes, however, they are as large as or larger than the chromosomes, and may bear such a striking likeness to them that there may appear to be as many as six or seven chromosomes (fig. 36). When the spindle is completely formed, these bodies may still be present on fibers connected with the spindle or nucleolus. Only very small ones, however, have been seen on the spindle, so that when the spindle is formed these bodies, which usually stain lighter than the chromosomes, can be readily distinguished from them.

After the linin fibers have become connected with the centrosome, they increase in number. The centrosome then divides, and the two daughter centrosomes take positions at the opposite ends of the spindle (figs. 37 and 38). When the spindle is first formed, it may be at any angle to the longitudinal axis of the ascus, but as division proceeds, it takes a position which is approximately parallel to it. While this is taking place, a set of fibers makes its appearance outside the nucleus. These fibers radiate from the centrosome into the cytoplasm for a considerable distance.

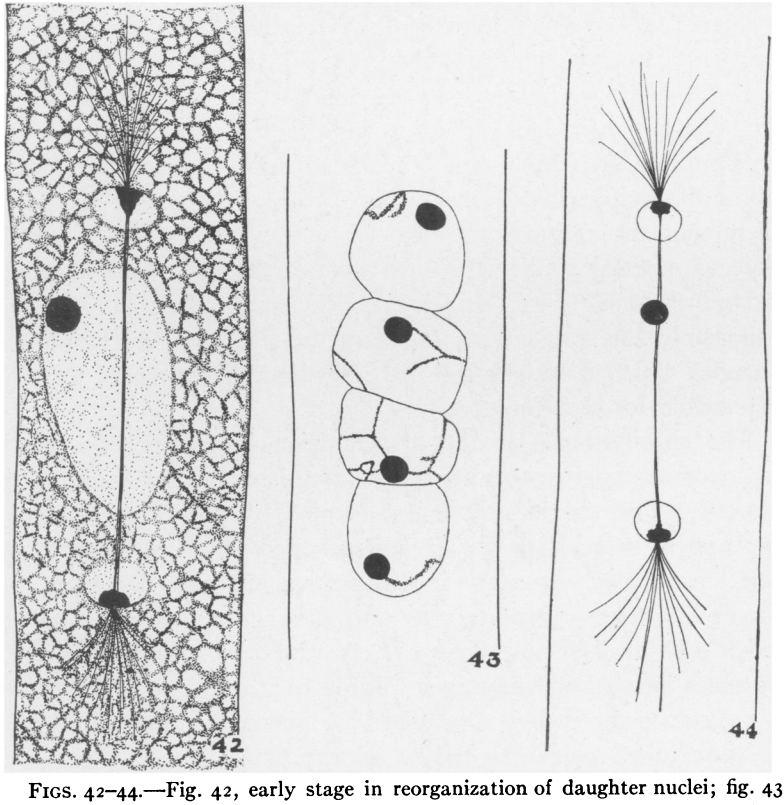
At metaphase five chromosomes are present on the spindle (fig. 38). Usually all of these appear to be somewhat elongated and have their longitudinal axis parallel to that of the spindle. Each of the five chromosomes divides transversely, but the divisions do not all take place at the same time, so that as division proceeds, anywhere from six to ten chromosomes may be counted on the spindle. Remembering that when the spireme segmented it gave rise to five elongated chromosomes which were probably bivalent, it would seem that this division probably separates chromosomes which were placed end to end on the spireme and can have nothing to do with the longitudinal split seen in the prophase. There appears to be nothing to indicate that the chromosomes which went into the fusion nucleus have persisted unchanged through the resting nucleus and the prophases of this division, and are the same as the chromosomes which are separated at metaphase. On the contrary, there would seem to have been every chance for an

exchange of material during synizesis, if not during the resting stage. The independence of unit characters in heredity would seem to favor the view that there may be an exchange of material between chromosomes, for if a given set of unit characters were permanently associated with the same chromosomes, we would expect to find different characters correlated much oftener than they are. If, however, as is generally assumed, the chromosomes are the part of an organism which is responsible for the transmission of hereditary characters, and if different chromosomes are not alike but responsible for different characters, it would be impossible for a promiscuous exchange of material between various chromosomes to occur without producing chaos. It would seem more likely that the chromosomes are so constituted that only certain kinds of material can be fitted into them, so that while chromosomes derived from different nuclei may exchange material which is responsible for similar sets of characters, they cannot exchange material which is responsible for one kind of character for that responsible for a different kind.

The chromosomes at the first division in *Lachnea* appear to approach the poles rather slowly, as anaphase is very abundant in sections. The ten chromosomes, formed by the division of the five seen at metaphase, are at first grouped at the equator of the spindle and give this stage a striking resemblance to metaphase. Finally, however, they separate into two groups of five, one of which goes to each pole (fig. 39). As the chromosomes approach the poles all of them may again divide (fig. 40). The two halves of a chromosome do not appear to be connected, but when division has just taken place the halves appear to be arranged in pairs, the constituents of which usually lie side by side on the spindle. It would seem from this that this division is due to a longitudinal splitting, and this may be connected with the splitting of the spireme seen in the prophase. A division of the daughter chromosomes as they approach the poles has been described in *Gallactinia succosa* by MAIRE (**27**) and GUILLIERMOND (**18**).

After the chromosomes have reached the poles, the fibers which connect the centrosomes continue to elongate until they become markedly bent. At the same time, breaks are formed on the

nucleus at each pole (fig. 41). Finally the groups of chromosomes break through the nuclear membrane, after which the fibers which connect the chromosomes straighten out and the groups of chromosomes are carried far beyond the limits of the nucleus (fig. 42). The nuclear membrane then breaks down. The nucleolus is left

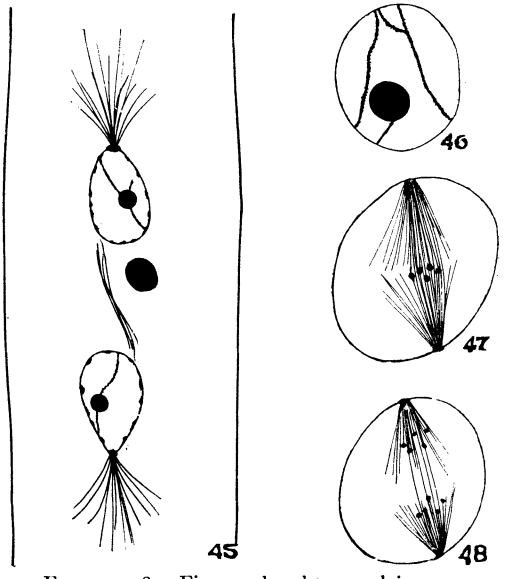


FIGS. 42–44.—Fig. 42, early stage in reorganization of daughter nuclei; fig. 43, four nuclei of an ascus in contact; fig. 44, late stage in reorganization of daughter nuclei after first division; all ×2800.

in the cytoplasm and finally disappears. Both the fibers which connect the centrosomes and those which radiate out into the cytoplasm frequently persist until after nuclear membranes have been formed around the daughter nuclei (fig. 45). Sometimes the groups of chromosomes are not separated so far, and in this case the daughter nuclei may reorganize in contact with each other.

Occasionally this may occur at both the first and second divisions (fig. 43).

When the two groups of chromosomes have reached the place where the daughter nuclei are to be reorganized, they lie at the ends of the fibers which connect the centrosomes and just below those which radiate out into the cytoplasm. A clear area then makes its appearance on the side of the chromosomes which is away from the radiating fibers (fig. 42), and a membrane is formed around this clear area (fig. 44). The centrosome appears to be on the nuclear membrane and can be distinguished until the nucleus grows considerably, but after a time it seems to disappear. When the nucleus is first formed, the chromosomes are still arranged in a group on that side of the nucleus which is near the radiating fibers. As growth proceeds this group gradually grows smaller, while masses of chromatin make their appearance on other parts of the nuclear membrane. The nuclei are usually pear shaped (fig. 45). This appearance suggests that the radiating fibers exert a pull on the nucleus.

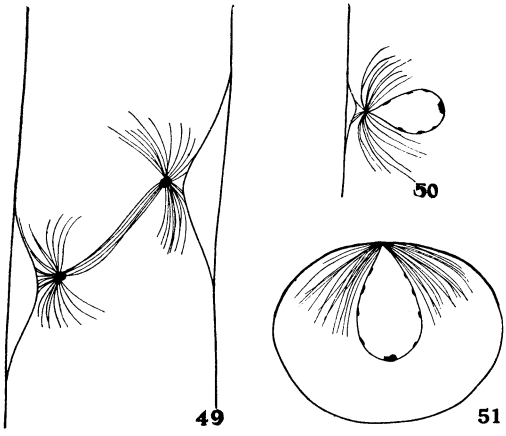


FIGS. 45–48.—Fig. 45, daughter nuclei reorganized; fig. 46, resting nucleus between first and second divisions; fig. 47, metaphase of second division; fig. 48, anaphase of second division; all ×2800.

The next division is homotypic, and shows no new features. The chromatin becomes arranged in a spireme (fig. 46) which gives rise to five chromosomes. These chromosomes are usually rather close together, and frequently they become aggregated in a rather dense mass. This phenomenon appears to be similar to the grouping of the chromosomes in the prophases of the divisions in

the ascogonium and ascogenous hyphae. The spindles of this division are similar to those of the first, and usually lie in a plane which is approximately parallel to the axis of the ascus, but, as HARPER (21) has shown, they may vary markedly from this position. At metaphase the five chromosomes divide and five pass to each pole. Telophase and the reorganization of the daughter nuclei appear to be entirely similar to the same processes as described at the end of the first division.

The third division is essentially like the second, except that the spindles are usually approximately at right angles to the axis of the ascus although, as HARPER (21) has shown, one of them may be parallel to the ascus wall. At telophase, when the masses of chromosomes have broken through the nuclear membrane, some of the fibers which radiate from the centrosome out into the cytoplasm appear to be connected to the plasma membrane around the ascus (fig. 49). Where this occurs, the plasma membrane is pulled in toward the groups of chromosomes as though the fibers which connect the groups of chromosomes with the plasma membrane, by contracting, were drawing the membrane and group of chromosomes together (fig. 49). As the groups of chromosomes approach the periphery of the ascus, the radiating fibers come to be bent backward; this may be due to the movement of the centrosomes. The nuclei reorganize in a manner similar to that described for the daughter nuclei at the end of the first division, except that a more pronounced beak is formed on the nucleus where the radiating fibers



FIGS. 49–51.—Telophase of third division; radiating fibers attached to plasma membrane; fig. 50, nucleus reorganized after third division; fig. 51, spore showing beginning of secondary thickening of wall; fibers still apparent; all ×2800.

are joined to the centrosomes (fig. 50). The plasma membrane around the ascus, which was pulled in where the radiating fibers were connected with it, has by this time very nearly resumed its normal position against the ascus wall (fig. 50). The nucleus which is still connected by fibers to the membrane is, by this means, drawn toward the periphery, and this may account for the beak and also for the further bending back which has taken place in the radiating fibers which were not connected with the membrane.

Since the fibers seem to exert a pull on both the plasma membrane and the nucleus, and to be bent as a result of the movement of the nucleus, it would seem that they must be relatively solid structures. This view is strengthened by their behavior during telophase in all three divisions. After the chromosomes have reached the poles, the fibers connecting the centrosomes continue to grow and become bent as though under tension. At the same time beaks are formed on the nuclei at both poles. This may be due to the pressure of the connecting fibers, or in part at least to a pull exerted by the fibers radiating into the cytoplasm, as in the case of the beaks formed on the eight nuclei.

HARPER (**19, 21, 22, 23**) has described the cutting out of the spores in *Erysiphe*, *Lachnea scutellata*, *Pyronema*, and *Phyllactinia*. According to this author, the fibers radiating into the cytoplasm fold back and fuse into a membrane which grows back until its edges meet at a point opposite the centrosome. FAULL (**12**) has studied spore formation in a number of Ascomycetes, and concludes that the spores are not cut out by a membrane formed of fused astral rays. According to him the spores are delimited by a limiting layer of protoplasm. On the site of this there is formed a plasma membrane about the spore, and another opposed to it lining the cavity in the epiplasm. The formation of these is probably preceded by a cleavage of the limiting layer. The exospore is formed between the two opposed plasma membranes. OVERTON (**31**) in *Thecotheus pelletieri* and FRASER (**14**) in *Humaria rutilans* describe the spores as delimited by the astral rays. In *Lachnea*, the first sign of the cutting out of the spore is the appearance of a delicate membrane at the outer limits of the recurved astral rays. This usually appears first around the centrosome

and then is formed progressively until it cuts out the spore. This membrane is apparently not formed by a fusion of the astral rays, for although it appears at their outer limit, after it is completely formed the rays are still present within the spore and are apparently as numerous as ever, and in shrunken material both the centrosome and astral rays may be drawn completely away from the spore membrane. Moreover, in *Lachnea* there do not appear to be enough fibers to fuse together to form a membrane, unless, as pointed out by FAULL (**12**), they become flattened out very considerably, and there is no evidence that such is the case. Where there are a large number of fibers, as in *Phyllactinia* (HARPER **23**), the fusion would be a much simpler matter, but that they are very numerous where the membrane appears, and disappear as it is formed, is not sufficient evidence that they fuse. It would be necessary to see the actual fusion to prove that the spore is cut out by a membrane formed of fused fibers. What part, if any, the centrosome and fibers take in the formation of the membrane is doubtful. The appearance of the membrane just outside of them suggests that they may have something to do with its position. On the other hand, sometimes even before the membrane is completely formed the centrosome may be within it and not in contact with it. Stages showing a spore partly cut out are relatively rare, which indicates that when the process is once begun it takes place rapidly. Miss FRASER (**14**) says that FAULL'S account of the cutting out of the spores "does not seem to satisfactorily explain either the persistence of the astral rays or the formation of the nuclear beak." In this connection it may be noted that in *Lachnea* the astral rays usually persist after both the first and second division, until the daughter nuclei are completely reorganized, and that beaks are frequently formed on the nuclei, although these are not so prominent as those on the nuclei of the spores.

During the early stages of the formation of the membrane, it appears to be simply a differentiated part of the cytoplasm, and it is difficult to determine exactly when a distinct wall is formed, but the wall appears to be produced on the site of the original membrane. After the wall has been formed around the spore, it begins to thicken (fig. 51). This process frequently commences in the

region around the centrosome, but it may begin at any point. After the wall has become thickened, it is easy to determine that it is a distinct wall, with plasma membranes on both sides of it. This is shown especially clearly in material which has been shrunken, when it is possible to find, side by side, cases in which all of the contents have a normal position, and others in which either the plasma membrane around the spore or the one lining the epiplasm is drawn away from the wall. At this stage the astral rays are still plainly visible.

The stage at which the nucleus retracts its beak and rounds up is somewhat variable, but it usually does not take place until after the formation of the wall. When the beak is withdrawn, the centrosome may be left in the cytoplasm, but more frequently it remains in contact with the nuclear membrane. In either case it finally disappears.

As the spore reaches its mature size the wall around it thickens and becomes the exospore.

Discussion

HETEROTYPIC MITOSIS

The method of reduction in the number of chromosomes in *Lachnea* is quite similar to that described in *Dictyota* (WILLIAMS **37**), *Fucus* (YAMANOUCHI **41**), and in a large number of the higher plants. The chromosomes are arranged end to end in the prophase of the heterotypic division, and there is no evidence of a parallel fusion of spiremes.

The reducing divisions in *Lachnea* are quite unlike those in *Phyllactinia* (HARPER **23**). This is perhaps not surprising in view of the great dissimilarity which, according to the work of DAVIS (**11**), YAMANOUCHI (**39**), and LEWIS (**25**), is shown by different genera of the Rhodophyceae. The great difference between the mitoses in *Lachnea* and *Phyllactinia* would certainly make it unsafe to carry any conclusions in regard to nuclear phenomena from one form to the other.

There is in *Lachnea* nothing resembling the double reduction described in some other Pezizineae by FRASER (**14**), FRASER and

WELLSFORD (15), and FRASER and BROOKS (16). This is in harmony with the view that there is no fusion in the ascogonium.

SEXUALITY

It is unnecessary to review here the history of our knowledge of the sexuality of the Ascomycetes, as this has been thoroughly done quite recently by HARPER (22, 23), OVERTON (31), and LOTSY (26); while the latest literature has been discussed by FRASER (16). The passage of the nuclei from the antheridium into the ascogonium of *Pyronema confluens*, as reported by HARPER (22) and confirmed by CLAUSSEN (8), would seem to have established the view that the antheridium and ascogonium are to be regarded as sexual organs, even though the antheridium may be functionless or lacking in other cases. DANGEARD'S (10) failure to find a passage of nuclei from the antheridium into the ascogonium of *Pyronema confluens* may be due, as BLACKMAN and FRASER (3) suggest, to his having worked on a different form from that observed by HARPER and CLAUSSEN. The writer has found that the antheridia may behave differently in different strains of *Pyronema confluens*. In one (BROWN 5), the antheridia never fused with the trichogyne, while in a strain of *Pyronema* (*confluens*) *omphalodes*, obtained through the kindness of Dr. F. J. SEAVER, the antheridium at the proper stage, as has been figured by him (SEAVER 34), can be readily seen fused to the trichogyne. The two strains, moreover, show differences in the conditions under which they can be grown. It is interesting in this connection that VAN TIEGHEM (35) has shown that under cultural conditions the antheridium of *Pyronema confluens* may be normal, rudimentary, or absent, while the ascogonium develops normally.

Since recent work has shown that the fusion of nuclei is the essential part of fertilization, the discussion of the sexuality of the Ascomycetes has naturally centered around the nuclear fusions. In the simple forms *Eremascus fertilis*, *Endomyces magnusii* (GUILLIERMOND 18), and *Dipodascus albidus* (JUEL 24), the antheridium and oogonium fuse and give rise at once to a single ascus. In *Eremascus fertilis* the antheridium and oogonium are uninucleate, and in all three cases the primary nucleus of the

ascus is formed by the fusion of a nucleus from the oogonium and one from the antheridium.

Among the Erysibaceae, HARPER (**19, 20, 23**) has described the fusion of a uninucleate antheridium and oogonium in *Sphaerotheca humuli*, *Erysiphe communis*, and *Phyllactinia corylea*. According to HARPER, the male and female nuclei fuse in the oogonium, and this is followed later by a second nuclear fusion in the ascus. DANGEARD (**9, 10**) has studied the development of *Sphaerotheca humuli* and *Erysiphe*, and denies the presence of a fusion in the oogonium.

BARKER (**2**) has described the fusion of an antheridium and oogonium in *Monascus*. He did not find a fusion of nuclei in the oogonium, but attributed this to his failure to get the proper stages. SCHIKORRA (**33**) has also described the fusion of an antheridium and oogonium in *Monascus*, but does not find any fusion of nuclei except the one in the ascus.

Among the Pezizineae the fusion of nuclei in pairs in the ascogonium has been described in *Pyronema confluens* (HARPER **22**), *Humaria granulata* (BLACKMAN and FRASER **3**), *Lachnea stercorea* (FRASER **13**), *Ascobolus furfuraceus* (WELLSFORD **36**), *Ascophanus carneus* (CUTTING **7**), and in the vegetative hyphae in *Humaria rutilans* (FRASER **14**). In all of the above cases a second fusion is described in the ascus. CLAUSSEN (**8**), however, after studying *Pyronema confluens*, has concluded that there was no fusion of nuclei in the ascogonium. BROWN (**4**) came to the same conclusion in regard to a form of this species in which the antheridium did not fuse with the trichogyne. This conclusion was confirmed by the behavior of the chromosomes in the ascus. In *Lachnea* it would seem to be quite evident that there is no fusion of nuclei in the ascogonium, but there are appearances connected with division which may be readily mistaken for fusions. During prophase, when the nuclei are of course large, the massing of the chromosomes into a nucleolus-like group gives an appearance much like a fusion nucleus, while the reorganization of fusing nuclei in contact simulates fusing nuclei rather closely. Similar appearances have been seen by the writer (BROWN **4**) in *Pyronema*. In view of these facts and the increasing amount of negative evidence, it would

seem necessary to study the structure and behavior of the nuclei in the ascogonium quite closely before deciding that there is a fusion of nuclei in the ascogonium of any of the Pezizineae, and it is worthy of note that divisions have not been described in any of those mentioned above in which such a fusion is said to occur. This is particularly true of such an aberrant case as the occurrence of a second fusion following the sexual one in the life history of the same plant.

ALTERNATION OF GENERATIONS

When HOFMEISTER used the term alternation of generations, he of course did not know of the alternation of the haploid and diploid number of chromosomes, but meant the alternation of two kinds of plants, one of which bore sexual and the other asexual reproductive bodies. Since the significance of nuclear phenomena has come to be better understood, many writers have been inclined to use the term alternation of generations as synonymous with the alternation of the haploid and diploid number of chromosomes, but the question may be asked as to whether the two things always necessarily coincide. If we take the cases of *Alchemilla* (MURBECK **30**), which has an embryo sac with the diploid number of chromosomes, and *Nephrodium* (YAMANOUCHI **40**), which produces sporophytes with the haploid number, there is of course no alternation of the haploid and diploid number of chromosomes, but from the standpoint of phylogeny there is an alternation of two kinds of plants. In *Coleochaete*, where the zygospore divides to form a number of cells which produce zoospores, the cells formed from the zygospore may be regarded as an intercalated asexual phase, but reduction takes place at the first division of the zygospore (ALLEN **1**). Here there would seem to be, as FARMER has suggested, a sporophyte which normally has the same number of chromosomes as the gametophyte. In the red alga *Griffithsia bornetiana*, LEWIS (**25**) thinks that the sexual plants and the mass of carpospores constitute an antithetic alternation of generations, while the sexual and tetrasporic plants represent the alternation of an homologous phase. According to this interpretation, the diploid number of chromosomes would extend through two distinct phases.

It seems probable that the ascogonium in some of the ancestors of *Lachnea scutellata* was fertilized, and that this ended the gametophytic phase and initiated the sporophytic, which ended in the production of spores. According to the interpretation usually applied to the delayed nuclear fusion in the rusts, the above interpretation would hold even if nuclear fusion was delayed, as CLAUSSEN (**8**) claims to be the case in *Pyronema confluens*, until the formation of the ascus.

From a phylogenetic standpoint, it would seem reasonable, therefore, in the case of *Lachnea scutellata* to regard the stages from the spore to the ascogonium as gametophytic, and those from the formation of the ascogenous hyphae to the production of spores as sporophytic. The diploid number of chromosomes exists, however, only in primary nucleus of the ascus. Even if we should adopt DANGEARD'S (**10**) interpretation, and regard the ascus as an oogonium, the third division in the ascus, which shows the haploid number of chromosomes, would still appear to belong to the sporophyte. It would seem advisable, therefore, in the case of *Lachnea scutellata*, as in those previously mentioned, to distinguish between the alternation of generations and the alternation of the haploid and diploid number of chromosomes. The gametophyte is usually regarded as beginning with the spore mother cell, but if the ideas brought forward here are correct, this can hardly be the case in *Coleochaete* or *Lachnea scutellata*, and it would seem better to think of it as beginning with the spore.

Summary

The mature ascocarp of *Lachnea* is disk-shaped. The hymenium forms the upper surface, while the rim and lower surface are covered by a thick-walled cortical layer. The center is composed of rather loosely interlacing hyphae.

The ascogonium is the penultimate cell of a row of about nine.

The ascogonium is early surrounded by vegetative hyphae, the outer of which form the first part of the cortex, while those around the ascogonium remain active and give rise on one side to more of the cortex and on the other to hyphae which will produce paraphyses. When a part of the cortex is once formed, the develop-

ment of the hyphae composing that part ceases. The cells between the cortex and hymenium, however, remain active and add to the cortex and to the hyphae which produce paraphyses.

The ascogenous hyphae are large and branch profusely. At the ends of these are formed typical hooks, consisting of binucleate penultimate and uninucleate ultimate and antepenultimate cells. The two nuclei of a penultimate cell may fuse to form the nucleus of an ascus, or they may divide and give rise to the four nuclei of another hook. The uninucleate ultimate cell usually grows down and fuses with the antepenultimate cell, after which the two nuclei may give rise to the nuclei of another hook, or they may fuse to form an ascus.

When the hymenium is first formed, it is covered by the younger setae of the cortex, but as its diameter is increased and its level raised by the multiplication of the number of asci and paraphyses, it comes to be exposed.

No fusion of nuclei was observed in either the ascogonium or ascogenous hyphae, except where two nuclei fuse to form the primary nucleus of an ascus.

The nuclei of the ascogonium and ascogenous hyphae appear to be entirely similar except for size, and the same number of chromosomes, five, persists throughout their divisions. When the chromosomes are first formed, they are frequently grouped in a mass resembling a second nucleolus. The chromosomes become connected with a centrosome which was not apparent during the resting stage. This centrosome divides, and the two daughter centrosomes come to be situated at the poles of the spindle. At metaphase the five chromosomes divide, and at anaphase five pass to each pole. The daughter nuclei are usually organized at some distance from each other, but sometimes they are so close together that they resemble fusing nuclei.

The first division in the ascus is heterotypic. Synizesis is produced by the contraction of a single spireme. After synizesis the spireme splits longitudinally. The two halves come together again, after which the spireme contracts considerably and segments into five elongated chromosomes. A centrosome makes its appearance on the nuclear membrane and becomes connected with the chromo-

somes by linin fibers in the nucleus. The centrosome divides and the daughter centrosomes come to be situated at the poles of the spindle. The chromosomes divide transversely. As they approach the poles they appear to split longitudinally. The second and third divisions in the ascus are similar to those in the ascogonium.

The spore wall does not appear to be formed by the fusion of astral rays.

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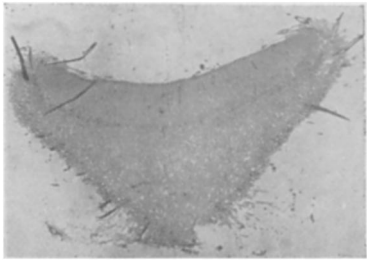

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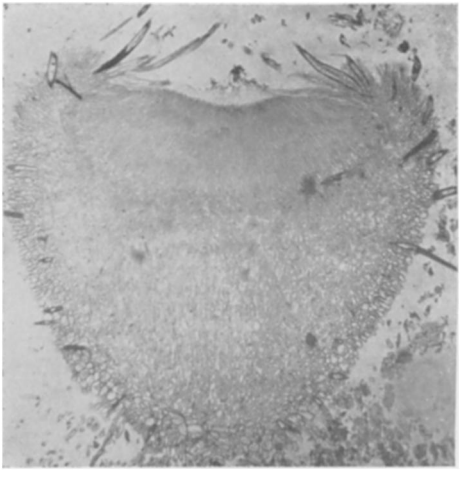

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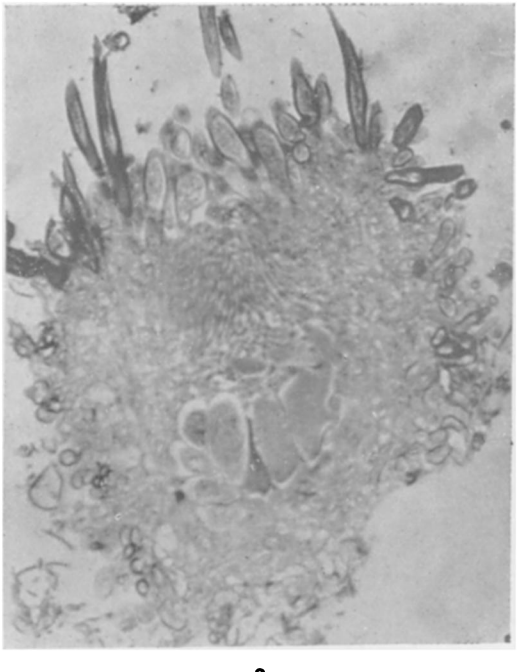

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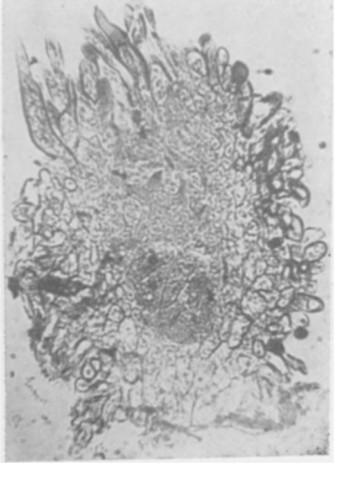

2

BROWN on LACHNEA

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EXPLANATION OF PLATE IX

FIG. 1.—Vertical section of mature ascocarp.

FIG. 2.—Vertical section of young ascocarp, showing young archicarp surrounded by comparatively few vegetative hyphae.

FIG. 3.—Vertical section of older ascocarp, showing ascogonium giving off ascogenous hyphae.

FIG. 4.—Vertical section of ascocarp, showing early stage in formation of hymenium.